THE JUVENILE HORMONE. II. ITS ROLE IN THE ENDOCRINE CONTROL OF MOLTING, PUPATION, AND ADULT DEVELOPMENT IN THE CECROPIA SILKWORM

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In the history of every hormone there is a more or less prolonged period when the factor is recognizable only as a certain "activity" within a living system. Progress at this stage is largely dependent on the development of a method of biological assay which is simple, selective, and quantitative.

In the case of the juvenile hormone of insects, the pioneeering studies that led to the discovery of the hormone simultaneously directed attention to a method for its assay. This method, as developed by Wigglesworth (1936, 1948, 1958), is performed on mature nymphs of *Rhodnius*. In brief, a fifth instar nymph is given a blood meal to provoke the molt to the adult stage. If one now implants active corpora allata (or, by parabiosis, transfuses blood containing juvenile hormone), the nymph transforms into an adult which retains certain nymphal characteristics. By the use of this assay, Wigglesworth (1940) found the corpus allatum of *Rhodnius* to be active in the immature nymph, inactive in the mature nymph, and active again in the adult.

Numerous investigators, following Wigglesworth's lead, have utilized the "larval assay" in testing the endocrine activity of corpora allata. The literature includes studies of the following genera: *Bombyx* (Bounhiol, 1938; Fukuda, 1944; Ichikawa and Kaji, 1950), *Dixippus* (Pflugfelder, 1939, 1958), *Tenebrio* (Radtke, 1942), *Galleria* (Piepho, 1942, 1950b), *Melanoplus* (Pfeiffer, 1945), *Drosophila* (Vogt, 1946), *Gryllus* (Poisson and Sellier, 1947), *Oncopeltus* (Novák, 1951), and *Calliphora* (Possompès, 1953). The conclusions derived in all these studies have confirmed the fact that the corpora allata undergo substantial changes in activity during the course of metamorphosis.

At the Harvard laboratory we also have tried to make use of the larval assay in testing for juvenile hormone. A survey of all of our experiments performed during the past fifteen years fails to reveal a single instance in which the implantation of active corpora allata interfered with the transformation of fifth stage Cecropia larvae into normal pupae. For reasons that are not fully understood, the larval assay does not work in the case of the Cecropia silkworm.

Solution of our problem came from an unexpected direction. As described in the previous paper of this series, the "pupal assay" was accidentally discovered in 1947; unlike the larval assay, it proved to be an extremely sensitive test for juvenile hormone (Williams, 1952a, 1959).

In the present study the pupal assay has been used as a principal tool in a study

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of the role of juvenile hormone in the growth and metamorphosis of the Cecropia silkworm.

Material and Methods

The experiments were performed on larvae, pupae, and adults of the Cecropia silkworm, and on pupae and adults of the Cynthia silkworm. In addition to methods described previously, the following procedures were utilized.

1. *The pupal assay for juvenile hormone*

Pairs of corpora allata-corpora cardiaca complexes were excised from larval, pupal, or adult donors, and placed in Ringer's solution. In the early experiments the corpora allata were dissociated from the closely attached corpora cardiaca. This tedious maneuver proved inconsequential (Williams, 1959) and was generally omitted in later experiments.

The glands were tested for endocrine activity by implanting them into the abdomens of female Cecropia pupae that had been stored at 6° C. for at least four months. For reasons considered below, pupae that had already initiated adult development were not suitable as test animals. The implantation was accomplished as follows:

Each test pupa was anesthetized with carbon dioxide, and a disc of integument excised from the tip of its abdomen. By means of forceps, one to eight corpora allata were placed among the strands of fat-body deep inside the abdomen. Crystals of an equal part mixture of phenylthiourea and streptomycin sulfate were spread in the wound, along with sufficient Ringer's solution to fill the body cavity. The wound was capped with a plastic window which was sealed in place with melted wax. Finally, the pupa was returned to air and stored at 25° C.

Within a few days the wound was healed by a deposit of blood cells on the plastic window and the centripetal spread of the simple epidermis from around the margins of the wound. Adult development was ordinarily initiated after about ten days; the zero day of development was recognized by the beginning of the retraction of the regenerate epidermis underlying the plastic window (Schneiderman and Williams, 1954).

On about the fifth day of development the first indication of a positive test for juvenile hormone was evident under the window. Here one could witness the formation of a new pupal cuticle which soon became tan and sclerotized. A few days later, the old pupal cuticle became thin and crisp, due to a precocious activation of the molting-fluid and the localized resorption of endocuticle (Passonneau and Williams, 1953). Commonly, one could detect the discharge of a white sludge of meconium into the molting-fluid—an event that has rarely been observed in the course of normal metamorphosis.

Development was allowed to proceed for a total of twenty-one days or until the molting-fluid was partially resorbed. The pupal exuvia was then peeled away with forceps. The insect was immersed in Ringer's solution and subjected to detailed external and, in many cases, internal examination.

A positive test for juvenile hormone is signaled by the preservation of pupal characters (Williams, 1952a, 1956, 1959)—a finding which will be considered in detail in the section on Results.

2. *Excision of larval corpora allata*

Matched pairs of larvae were sacrificed and the corpora allata dissected from their heads, as previously described in the case of adult corpora allata (Williams, 1959, page 327). In certain experiments it was necessary to excise the corpora allata without killing the larval donors. By adaptations of a method suggested by Dr. William Van der Kloot (unpublished observations), a surgical approach through the underside of the neck was utilized, as follows:

A 3-cm. length of dowel was attached to a small base-board so that the dowel stood vertically above the perforated plate of the anesthesia funnel. The top end of the dowel was grooved to fit the dorsum of the larval head capsule. The larva was deeply anesthetized and placed in the anesthesia funnel so that the underside of the neck was stretched and flexed over the top end of the dowel. The head capsule was held in the groove by small clips so that the thorax and anterior abdominal segments hung vertically. In this way the blood was pressed from the neck region and the latter was flattened and essentially bloodless.

The ventral midline of the neck was lifted with forceps and a single V-shaped incision was made through the integument with microscissors. Under a dissecting microscope the operation was carried out through the incision, first on one side of the neck and then on the other. With blunt probes the muscles of the neck were pressed apart and the corpora allata located and excised.

At the conclusion of the operation the flap of skin was spread in place. The animal was stored in a capped cardboard container at 5° C. until the next day. It was then returned to room temperature and placed on a netted branch of wild-cherry leaves.

3. *Excision and transplantation of wing-discs*

The thorax plus first abdominal segment was isolated, opened along the mid-dorsal line, and spread and pinned under Ringer's solution. After the removal of the viscera, the two pairs of wing-discs were located between the body wall and the complex musculature of the meso- and metathorax.

Each disc, together with its peripodal sac, was trimmed away from surrounding structures and placed in a dish of insect Ringer. In some cases the dissection was continued by cutting away the peripodal sac. The discs were implanted under abdominal windows of previously chilled pupae, as described above.

Experimental Results

1. *Quantitative aspects of the pupal assay for juvenile hormone*

When a living, active corpus allatum is implanted into a test pupa, the gland survives and becomes the site of synthesis and secretion of juvenile hormone. If several active glands are implanted, a corresponding number of synthetic centers are established. So, in theory, one should be able to vary the rate of secretion and accumulation of juvenile hormone by varying the number of implanted glands. The developmental reactions of the test animal should then reflect the quantitative aspects of the pupal assay.

In experiments of this type, advantage was taken of the high activity that one routinely observes in tests of the corpora allata of freshly emerged Cecropia moths

(Williams, 1959). Four (two pairs) adult corpora allata were implanted into each of 24 test pupae; a second group of 24 pupae each received only a single implant (one-half pair). The hosts were then placed at 25° C. to await the onset of development.

Three to four weeks later, a spectacular difference was evident between the two groups of animals. The individuals that received the four implants showed a generalized inhibition of adult differentiation, as signaled by the formation of a new pupal cuticle throughout broad areas of head, thorax, and abdomen. Indeed, some of these animals could properly be described as second pupal stages in which only

TABLE I

Developmental status of previously chilled Cecropia pupae after receiving implants of corpora allata. The endocrine activity of the implants is scored in intensities ranging from zero to five

Intensity of reaction	Anatomical characters			
	Head	Thorax	Abdomen	Internal anatomy
0	Adult	Adult	Adult	Adult
1	Normal adult, except for small facet-free crescents in eyes*	Adult with pupal cuticle occasionally in midline of prothoracic tergum*	Adult except for small zone of pupal cuticle at base of genitalia*	Adult
2	Normal adult, except for facet-free crescents in eyes	Pupal cuticle in midline of tergum	Zones of pupal cuticle anterior to intersegmental membranes; genitalia slightly arrested with localized pupal cuticle	Adult with slight suppression of gonad development
3	Covered with pupal cuticle with pubescence only on frons; antennae show incomplete barbs and pupal cuticle locally on shaft	Large zones of pupal cuticle on tergum and legs; wings show scales, but incomplete pigmentation	Generally covered with pupal cuticle showing sparse pubescence; genitalia very inhibited and covered with pupal cuticle	Gonads show considerable arrest; flight muscles show only early development; gut retains pupal configuration; prothoracic glands persistent
4	Pupal cuticle on head, antennae, and palps; antennae show only traces of segmentation or of subdivision into barbs	Pupal cuticle throughout with only localized islands of pubescence; wings generally white, fleshy and friable	Pupal cuticle throughout; genitalia show no development	Only traces of development in gonads, gut, and muscles; fat-body friable and in chunks; prothoracic glands persistent
5	Pupal throughout except for small pigmented eyes	Pupal cuticle throughout; sometimes also at base of wings and around margins of forewings	Wholly pupal	Pupal; prothoracic glands persistent

* Pupal cuticle forms in all zones where the integument has been injured.

traces of adult characteristics had been differentiated. By contrast, the pupae that had received only a single corpus allatum ordinarily developed into adult moths which showed few abnormalities except for the formation of a new pupal cuticle under the plastic window where the pupal integument had been excised in the implantation procedure.

These results have been duplicated and extended on a large scale during the past fourteen years. Under most conditions and circumstances, the standardized pupal assay has proved to be a sensitive, selective, and semi-quantitative test for the concentration of juvenile hormone.

Under the standardized conditions of the assay, each concentration of juvenile hormone ordinarily produces a certain pattern of inhibition. In practice, it was possible to subdivide the intensities of the reactions into six categories. This scoring system, summarized in Table I, permitted the pupal assay to be used in a study of corpus allatum activity at successive stages in the life history of the Cecropia silkworm.

2. *Endocrine activity of corpora allata*

A. Larval corpora allata

Corpora allata were removed from matched pairs of Cecropia larvae at stages ranging from the middle of the third instar to the end of the fifth instar. The two pairs of glands were, in each case, implanted into a single female test pupa, as described under Methods.

Before considering the results of the assays, it is worth recalling that the growth and metamorphosis of the corpora allata are synchronized with the growth and

TABLE II

Activity of Cecropia corpora allata implanted into chilled Cecropia pupae (2 pairs of glands into each test pupa)

Stage of donors	No. of tests	Intensity of hormone reaction						Average Index*
		0	1	2	3	4	5	
3d instar	5	1	2	0	2	0	0	1.6
Late 3d	2	0	1	1	0	0	0	1.5
3d molting	9	0	3	5	1	0	0	1.8
Early 4th instar	11	0	2	2	6	1	0	2.5
4th	6	1	1	2	2	0	0	1.8
Late 4th	6	1	2	2	1	0	0	1.5
4th molting	10	1	5	2	2	0	0	1.5
Early 5th instar	12	0	4	0	7	1	0	2.4
5th	19	4	9	3	3	0	0	1.3
Late 5th	13	2	2	6	3	0	0	1.8
1st day spinning	19	2	15	2	0	0	0	1.0
2nd day spinning	12	2	10	0	0	0	0	0.8
Finished spinning	7	0	6	2	0	0	0	1.1
Early prepupa	10	4	6	0	0	0	0	0.6
Fresh pupa	10	10	0	0	0	0	0	0
Unchilled pupa	7	7	0	0	0	0	0	0
Chilled pupa	29	29	0	0	0	0	0	0
1–2 day adult development	5	5	0	0	0	0	0	0
9–11 day	6	6	0	0	0	0	0	0
14th day	18	13	1	2	2	0	0	0.6
17th day	4	3	1	0	0	0	0	0.25
Adult (freshly emerged)	24	0	0	2	5	12	5	3.8

* For definition, see text.

metamorphosis of the animal as a whole (for review see Pflugfelder, 1958). And since four glands were routinely implanted into each test pupa, the assay did not compensate for the changing mass of endocrine tissue. What the pupal assay recorded was the endocrine activity of the four implants, irrespective of size, rather than the activity per unit mass of endocrine organ.

Over a period of several years a total of 131 assays were performed on larval corpora allata. In the results tabulated in Table II, a certain scatter is observed in the activity recorded at each stage. In order to derive an overall index of activity

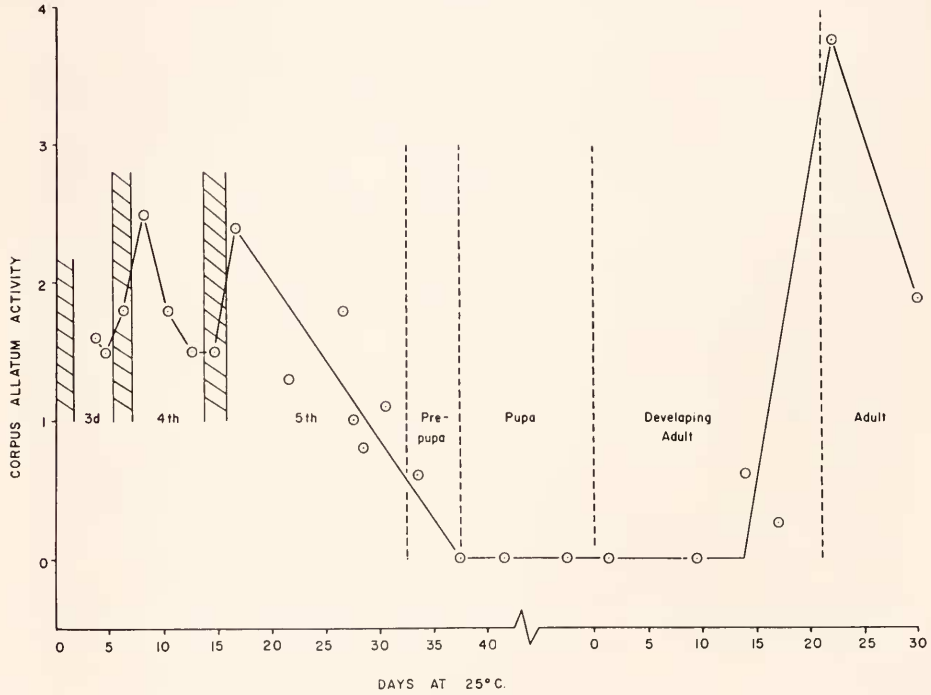

FIGURE 1. Changes in the endocrine activity of the corpora allata of the Cecropia silkworm during the third, fourth, and fifth instars of larval life and during metamorphosis. The average indices of corpus allatum activity (derived from "pupal assays") are plotted as a function of the stage of development of the donor animals. The discontinuity in the x-axis signals the storage of donor pupae at 6° C. for 10 to 20 weeks; the cross-hatched zones correspond to the periods of larval molting.

at each stage, a zero response was counted as zero, a one response as one, a two as two, and so on. The total was then divided by the number of experimental animals to yield an "average index" of corpus allatum activity at each stage.

In the plot of these average indices in Figure 1, attention is first directed to what appears to be a cycle of corpus allatum activity during the third and fourth larval instars. Their endocrine activity is apparently minimal just prior to the molt, maximal shortly after the molt, and subject to a steady decline during the instar itself. As illustrated in Figure 1, this decline is particularly striking during the final (fifth) larval instar.

Late in the fifth instar, when the activity of the corpora allata has declined to a critically low level, a dramatic change occurs in the silkworm's behavior. It ceases to feed, empties its gut, and then begins a two-day period of spinning. This impressive change in behavior is the earliest sign of approaching pupation.

B. Prepupal corpora allata

The onset of the prepupal stage is signaled by a detachment and retraction of the epidermis from the larval cuticle and by the initiation of the cytological events which, during a period of five days, transform the larva into a pupa. In Figure 1 we note the surprising finding that the corpora allata show a low but definite activity during the prepupal period—a result which will be considered below in further detail.

C. Pupal corpora allata

The decline in corpus allatum activity continues during the prepupal period. Then, immediately after the pupal ecdysis, the glands for the first time are found to be completely inactive.

As illustrated in Figure 1, the inactivity of pupal corpora allata persists during the entire pupal period, irrespective of whether the pupae are stored at high or low temperatures. In my entire series of assays I have never obtained a positive test for the pupal corpora allata of Cecropia, Polyphemus, Cynthia, or any other lepidopteran. In a univoltine species such as Cecropia, the pupal stage extends from midsummer until the following spring. So, during a period of about eight months, the corpora allata are apparently inactive.

D. Corpora allata during adult development.

Adult development, once initiated, requires three weeks at 25° C. The corpora allata continue to be inactive during the first two weeks of this period. On the fourteenth day of development one begins to record renewed activity in the glands of a certain proportion of individuals (Table II and Fig. 1). Within the final week of adult development the corpora allata recover their endocrine activity. Indeed, in the pupal assay the glands of freshly emerged male or female moths show higher activity than at any other stage in the life history.

In the absence of functional mouth-parts, the Cecropia moth lives for only 7 to 10 days at 25° C. During this brief period the glands of most individuals undergo a marked decline in activity to a level approximately one-half of that a week earlier.

3. *The role of juvenile hormone in pupation*

As noted in Section 2B, the corpora allata show a low but definite activity during the prepupal period. This is an extremely surprising result since the prepupal stage is distinguished by metamorphic changes on an unprecedented scale. The experiments, about to be described, were designed to test the physiological significance of the low concentration of juvenile hormone that is apparently present during the prepupal period.

In an initial series of experiments performed in collaboration with Dr. William H. Telfer, the corpora allata were excised from a group of eight Cecropia larvae early in the fifth instar. The loss of the glands seemed inconsequential. Feeding and growth continued and two weeks later the animals spun normal cocoons. But, within the following ten days, only two of the eight animals transformed into normal pupae. The other six formed strange creatures in which a considerable number of tissues and organs had overleaped the pupal stage by undergoing precocious adult differentiation.

One of these animals is illustrated in Figure 2. The head shows the pigmented, faceted, compound eyes of the adult. The antennae exhibit the segmentation and

FIGURE 2. By excision of the corpora allata during the final larval instar, this animal was caused to undergo pupation in the absence of juvenile hormone. A large number of larval tissues and organs have undergone precocious adult differentiation without traversing the pupal stage.

subdivisions characteristic of early adult development. The thorax is covered with a mixture of rugose pupal cuticle and smooth cuticle of the adult type. The adult patagia have developed at the base of the fore-wings. The sclerotization of the thoracic tergum is adultoid. The thoracic pleura and sternum are covered for the most part by a smooth, adult-type cuticle. The legs show segmentation and the differentiation of tarsal claws and pulvilli. The proximal ends of the wings are covered by adult cuticle. The cuticle of the abdomen is wholly pupal, except in the immediate region of the genitalia; the latter are represented, not by imaginal discs, but by miniature adult genitalia which show an early elaboration of the various valves and adult structures. Dissection revealed that the fat-body was similar to that of a pupa after the initiation of adult development. Moreover, the ovaries

TABLE III

Effects of removal of corpora allata from fifth instar Cecropia larvae

State at time of operation	No. of animals	Results	
		Normal pupa	Pupal-adult
Young 5th instar	3	0	3
Mid 5th instar	9	5	4
Late 5th instar	5	2	3
Spinning	13	12	1

showed the differentiation of ovarioles to a stage corresponding to that encountered in early adult development.

The experiment was repeated on a larger scale utilizing animals at successive stages in the fifth instar. The results in Table III record the production of pupal-adult mixtures in all three animals from which the corpora allata were extirpated early in the fifth instar. Excision in the mid or late fifth instar gave rise to mixed forms in about half the animals. And when the operation was further postponed to the period of spinning, only one of thirteen animals developed into a mixed form.

These findings suggest that the juvenile hormone plays a definite role in the endocrine control of pupation. Evidently, the low concentration of juvenile hormone in the mature larva and prepupa is necessary to prevent the precocious adult differentiation of larval tissues and organs.

4. *Precocious adult differentiation of larval wing-discs*

Wing-discs were excised from fourth and fifth instar Cecropia larvae and implanted into previously chilled Cynthia pupae just before the latter initiated adult development. In this manner the discs were exposed to the ecdyson of the developing host under conditions where juvenile hormone was absent. After the hosts had completed adult development, the implants were recovered and subjected to detailed examination.

As is true of implants of all epidermal tissues, the implants took the form of cyst-like structures with the integumentary surface facing inward. Each cyst was cut open and a record made as to whether it had formed a rugose pupal cuticle or a smooth, scale-covered cuticle of the adult type.

TABLE IV

Metamorphosis of larval wing-discs implanted into previously chilled pupae

Initial status of discs	No. of discs	No. of hosts	Differentiation of implants		
			Pupal	Pupal-adult	Adult
4th instar	20	10	5	8	7
Early 5th	2	2	0	2	0
Late 5th	9	4	4	1	5
Spinning 5th	4	2	0	4	0

The results are summarized in Table IV. Of the 35 wing-discs that were studied, 12 showed adult cuticle, 9 showed pupal cuticle, and 15 showed a mixture of pupal and adult cuticle. Certain cysts showed the differentiation of the articular sclerites distinctive of the adult wings.

It is noteworthy that precocious adult differentiation was readily obtained in wing-discs as early as the fourth instar. Consequently, when exposed to ecdyson in the absence of juvenile hormone, these implants omitted the fifth larval instar as well as the entire pupal stage. Here again we see that a certain low concentration of juvenile hormone is prerequisite for the transformation of a Cecropia larva into a normal pupa.

These results are reminiscent of Schaller's (1952) finding that larval honeybees undergo precocious adult development when decapitated prior to pupation. Moreover, Nayar (1954) has reported that pieces of larval integument undergo precocious adult differentiation when transplanted to pupae of *Ephestia, Galleria,* or *Pieris.* Here again we see that in many species a certain low concentration of juvenile hormone is necessary for the normal transformation of a larva into a pupa. This fact has long been suspected (Piepho, 1951; Wigglesworth, 1954, 1959; Schneiderman and Gilbert, 1959; Novák and Červenková, 1960), but is documented for the first time in the present investigation.

Discussion

1. *Juvenile hormone and adult development*

The experimental results direct attention to a prolonged period during which the corpora allata appear to be totally inactive in the secretion of juvenile hormone. This period begins immediately after pupation and continues throughout the entire pupal stage. Since the prothoracic glands are also inactive at this same time (Williams, 1952b) the pupal stage is characterized by subthreshold titers of both ecdyson and juvenile hormone.

After eight months of pupal diapause, the prothoracic glands recover their endocrine activity. Ecdyson is secreted and adult development begins. Meanwhile, the corpora allata continue to be inactive—a condition which persists throughout the first two-thirds of adult development.

The pupal assay derives its sensitivity from the fact that the early phase of adult development takes place only if juvenile hormone is absent. If the hormone is supplied by the implantation of active corpora allata, the net result is to block adult differentiation and to promote the formation of a second pupal instar. In effect, we duplicate the endocrine conditions peculiar to larval life in that ecdyson and juvenile hormone are caused to act side-by-side. The pupa molts into a further pupal instar just as, under the same circumstances, the larva molts to a further larval instar.

2. *Juvenile hormone and pupation*

The endocrine stimulus for pupation is the action of ecdyson in the presence of a low but finite titer of juvenile hormone. If the corpora allata are excised so that the larva approaches pupation in the absence of juvenile hormone, then a large number of larval tissues overleap the pupal stage and undergo precocious adult develop-

ment. The same result was observed when larval wing-discs were subjected to *in vivo* culture in the presence of ecdyson and the absence of juvenile hormone. Here again we see that the presence of a low concentration of juvenile hormone at the time of pupation serves as a brake on the precocious acting-out of the life-plan.

3. *Juvenile hormone and larval development*

According to the pupal assays, the corpora allata undergo a cycle of endocrine activity during successive larval instars. If the glands are extirpated so that an immature larva approaches a larval molt in the absence of juvenile hormone, the result, once again, is precocious metamorphosis in response to ecdyson (Bounhiol, 1938; Piepho, 1943; Fukuda, 1944; Williams, 1946).

In Figure 1 it is of particular interest to note that the corpora allata show a marked decline in activity during the intermolt period and are minimally active just prior to the molt itself. By virtue of the declining titer of juvenile hormone, one would anticipate some measure of progressive differentiation between successive larval instars.

This inference is confirmed by the striking heteromorphic changes (Snodgrass, 1954) which the Cecropia silkworm undergoes during the larval period. Thus, the first instar is jet black and covered with spinose tubercles; the second instar is yellow with black tubercles and spots; the third instar is greenish yellow with black spots and red, blue, and yellow spinose tubercles; the fourth instar is bluish green with pairs of red, yellow, and blue spinose tubercles; the fifth instar is bright green, shows distinctive red and yellow tubercles on the thoracic segments and a considerable reduction in the spines on all tubercles. In summary, each larval instar shows distinctive characteristics which, irrespective of the insect's overall size, permit one to recognize the individual instars with ease. It is no exaggeration to say that the magnitude of these changes between successive larval instars equals or exceeds those commonly encountered in hemimetabolous insects at the time of metamorphosis.

If larval heteromorphosis is attributable to the declining titer of juvenile hormone prior to the larval molts, then, by augmenting the concentration of juvenile hormone, one at least in theory should be able to obtain fifth stage larvae which retain the form of the first stage. Such experiments have not been performed. However, according to the unpublished observations of Dr. Judith Willis, the Cecropia silkworm, under certain environmental conditions, repeats the second, third, or fourth larval instar without change in form. Presumably, in all these cases, the corpora allata fail to undergo the normal decline in activity prior to the extra molt.

As pointed out by Snodgrass (1954), spectacular degrees of larval heteromorphosis are frequently encountered among predatory or parasitic species of insects, examples being known among the Neuroptera, Coleoptera, Strepsiptera, Lepidoptera, Hymenoptera, and Diptera. Evidently, the heteromorphic larval molts are here preceded by an even greater decline in corpus allatum activity than that encountered in the Cecropia silkworm.

4. *Theory of juvenile hormone action*

A comprehensive theory of the action of juvenile hormone must account for the following findings: (1) Ecdyson is a potent growth hormone either in the presence

or absence of juvenile hormone. (2) In all of the phenomena here under consideration, juvenile hormone is active only in the presence of ecdyson. (3) When juvenile hormone is absent, ecdyson promotes, not only the synthetic acts prerequisite for growth, but also the new synthetic acts that are necessary for metamorphosis. (4) When ecdyson acts in the presence of juvenile hormone, growth continues, but new synthetic acts are blocked to a degree proportional to the concentration of juvenile hormone.

From this summary we learn that the role of juvenile hormone is to modify the cellular reactions to ecdyson. It appears to do so by opposing progressive differentiation without interfering with growth and molting in an unchanging state. In some unknown manner, it blocks the de-repression and de-coding of fresh genetic "information" without interfering with the acting-out of information already at the disposal of the cells.

5. *The cytological actions of juvenile hormone*

Though we are unable to state how juvenile hormone controls the flow of fresh genetic information, certain clues may be derived from the data already at hand. Thus, in the case of the pupal-adult transformation, it is clear that the events which are sensitive to juvenile hormone take place at the very outset of adult development. So, if the implantation of corpora allata is delayed until the fifth day of the twenty-one days of adult development, it is already too late for juvenile hormone to have any effect. To be maximally effective, juvenile hormone must be present during the initiation of adult development; *i.e.*, during precisely the period when ecdyson is secreted by the prothoracic glands and acts throughout the pupa.

Evidently, the targets of juvenile hormone are certain very early events, including mitotic divisions, which are the normal cellular reactions to ecdyson at the outset of adult development. Whatever these cellular or subcellular events may be, we can state that they occur early, that they show a rapid loss of sensitivity to juvenile hormone, and that, if unopposed by juvenile hormone, they commit the cells to developmental reactions accompanied by metamorphosis.

It is worth recalling that, even at the outset of adult development, the various pupal tissues show a great range of sensitivities to juvenile hormone (Table I). Consequently, a cytological and cytochemical comparison between tissues of high and low sensitivity may yield additional information as to the mode of action of juvenile hormone. This approach seems particularly cogent in the case of the pupal epidermis whose sensitivity to juvenile hormone is vastly amplified at the site of an integumentary injury.

Summary

1. By means of a standardized "pupal assay" for juvenile hormone, the endocrine activity of the corpora allata was found to undergo large and systematic changes during the postembryonic development of the Cecropia silkworm.

2. In each of the larval instars that were studied, the glands are least active just prior to the larval molt and most active shortly after the molt. Larval molting therefore appears to take place in the presence of a declining titer of juvenile hormone—an endocrine situation which apparently permits the striking changes in

morphology and pigmentation which occur in successive larval stages of the Cecropia silkworm.

3. The corpora allata show low but definite activity at the time of pupation. If the glands are extirpated so that pupation occurs in the absence of juvenile hormone, many larval tissues overleap the pupal stage and undergo precocious adult differentiation. Therefore, the low concentration of juvenile hormone in the mature larva plays a definite role in the endocrine control of pupation.

4. The corpora allata are inactive throughout the entire pupal stage. This inactivity persists during the first two-thirds of adult development. During the final week of adult development, the glands recover their endocrine function and are maximally active by the time of emergence of the adult moth.

5. The absence of juvenile hormone proves to be an obligatory feature of the initial phase of adult differentiation. If active corpora allata are implanted into a pupa so that adult development is initiated in the presence of juvenile hormone, the pupa develops and molts into a creature which is a mixture of pupa and adult. The higher the titer of juvenile hormone, the more extensive is the preservation of pupal characters.

6. Juvenile hormone is effective in blocking adult differentiation only when it is present during the first five days of adult development. Consequently, the target of juvenile hormone appears to be certain early events which are the normal reactions to ecdyson at the outset of adult development. If unopposed by juvenile hormone, these events commit the cells to developmental reactions accompanied by metamorphosis.

7. A comprehensive theory is presented for the action of juvenile hormone in the Cecropia silkworm. According to this theory, juvenile hormone modifies the cellular reactions to ecdyson by opposing the de-repression, de-coding or acting-out of fresh genetic information prerequisite for progressive differentiation but not prerequisite for growth in an unchanging state.

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